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Advances in the Neurobiology of the Neuromuscular Junction

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Recombinant DNA technology and the development of the patch clamp technique for the recording of single channel activity have produced major advances in understanding the development of the neuromuscular synapse as well as functional aspects of neuromuscular transmission at the molecular level. In this abstract a short summary of the molecular mechanisms regulating neuromuscular synapse formation will set the background for understanding some congenital disorders of neuromuscular transmission. Finally, the mode of action of a neuromuscular blocking drug, succinyldicholine (suxamethonium) commonly used in anaesthesiological practice, will be introduced. For more comprehensive reviews, the reader is referred to some references are given below.

NMJ development

During development motor neurones begin to contact muscle cells when myoblasts are about to fuse into multinucleated myotubes. The latter express acetylcholine receptors (AChRs) constitutively along their entire length. AChRs in muscle are heteropentameric transmembrane proteins. At this stage they are composed of 2α, one β, one γ, and one δ-subunit, each encoded by a different gene, resulting in the subunit stoichiometry $\alpha_2\beta\gamma\delta$. Such AChR channels are termed ‚fetal'. Upon their activation by ACh, the fetal AChRs change their conformation to form ion channels in the muscle membrane for about 5 ms (burst duration, see below); the resulting inward current (about 3×10^{-12} A at –70 mV) depolarizes the myotube. AChR density combined with the high electrical input resistance of the myotube is sufficiently high that a single quantum of acetyl choline (ACh)s released from the motor nerve terminal can elicit an action potential from the earliest time of the contact. This is important for further muscle differentiation.

Within hours of the establishment of the neuromuscular contact AChRs begin to aggregate at the site of the contact, induced by Agrin secreted from the presynaptic nerve terminal and interacting with its receptor MuSK in the muscle membrane. Agrin is a heparan sulfate proteoglycan secreted from motor neurones. Originally, it was isolated by McMahan and colleagues from the basal laminae of electric organ of the electric ray *Torpedo*, based upon its ability of aggregate AChRs in cultured myotubes. To date, it has remained the only *bona fide* synaptic organizer molecule identified. Upon its secretion from motor nerve endings, Agrin binds to the basal lamina of the muscle fiber. Intriguingly, the synaptic basal lamina contains all molecular information to direct the regeneration of a presynaptic nerve terminal and a subsynaptic membrane upon nerve or muscle lesions. In animals lacking *agrin* or *musk* genes, NMJs do not form. Electrical impulse activity elicited in the muscle fiber by n.m transmission downregulates AChR subunit gene expression in non-synaptic fiber regions. Muscle nuclei located at the synapse, however, continue to transcribe α-, β-, and δ-subunit genes, and, in addition, begin to transcribe a new gene encoding the AChR ε-subunit. Thus, the subunit stoichiometry at the synapse switches from $\alpha_2\beta\gamma\delta$ to $\alpha_2\beta\varepsilon\delta$. The current passing through the latter (termed adult-type AChR channel) is about 4×10^{-12} A, and the apparent mean channel open time is about 1 ms. AChR gene expression selectively from nuclei at the synapse indicates that it is regulated by a factor from the nerve. Plausible candidates to mediate the neural control of synaptic AChR expression are certain isoforms of the neuregulins (NRG), a family of growth and differentiation factors, interacting

with ErbB receptor tyrosine kinases at the synapse. Another candidate is Agrin/MuSK which can stimulate synaptic AChR expression in the absence of motor neurones. Interestingly, MuSK induces the transcription not only of AChR subunit genes, but also of its own gene *musk* as well as of *erbB*, and it aggregates neuregulin derived from muscle in the muscle fiber basal lamina. In addition, it induces the synapse-specific expression of other synaptic components, such as voltage-gated Na^{+}-channels mediating action potentials, as well as structural end-plate components. Thus, Agrin acting through MuSK is the major organizer of subsynaptic differentiation, setting up multiple signalling loops feeding back to maintain gene transcription. It appears that expression of several of the synaptic genes (AChE, utrophin, AChRδ-subunit) are regulated via identical regulatory elements in their respective promoters, suggesting the involvement of similar intracellular signaling pathways.

Little is known on how Agrin/MuSK aggregates AChRs in the subsynaptic membrane. In response to activation by Agrin, MuSK, AChRs and several intracellular kinases are phosphorylated. The subsynaptic region of the muscle fiber is characterized by a number of membrane and cytoskeletal proteins thought to be involved in the aggregation of the AChRs and their anchoring to the cytoskeleton, and/or in endplate stabilization. A peripheral membrane protein, rapsyn, which interacts with the AChRs is indispensable for this process. Thus, animals lacking the *rapsyn* gene are not viable because they do not cluster AChRs. They do cluster MuSK, however, suggesting that MuSK-induced clustering of MuSK and of AChRs are regulated differentially, and that MuSK provides a scaffold to which other molecules can attach. Interestingly, the AChR itself is required for the assembly of a postsynaptic apparatus: in animals lacking AChRs, none of the subsynaptc components other than MuSK aggregate. Other components such as utrophin, and components of the DGC appear to be important for the mechanical stability of the NMJ.

Congenital myasthenic syndromes

A number of congenital myasthenic syndromes has been successfully linked to mutations in endplate components, most commonly to mutations in the AChR subunits, which in turn may affect the gating behaviour of the AChR channels. Channel behavior can be described by the following simple kinetic model:

$$A + R \underset{k^-}{\overset{k^+}{\rightleftarrows}} AR + A \underset{k^-}{\overset{k^+}{\rightleftarrows}} A_2R \underset{\alpha}{\overset{\beta}{\rightleftarrows}} A_2R^*$$

where an * denotes a channel in its open conformation. Flickering of the channel between A_2R and A_2R^* produces a burst of rapid openings and closures of the channel, whereas the magnitude of k_+ and k_- will dermine the frequency with which bursts occur. For example, different mutations in the AChR ε-subunit can affect the magnitude of the channel gating rate constants α or β relative to the dissociation rate constant k_-, thus prolonging or shortening the channel burst duration. Similarly, mutations of the α-subunit increase the affinity of ACh to the channel, again prolonging burst duration and increasing the probability for a burst. As a consequence, the endplate current is prolonged, allowing more Ca^{++} to enter the fiber and inducing fiber necrosis probably through the activation of intracellular, Ca^{++} dependent proteases. Mutations of the regulatory sequence responding to Agrin and NRG in the AChRε-subunit gene promoter lower the expression of the ε-subunit, and, thus, of synaptic AChR density. A concomitant increase in γ-subunit expression maintains n.m. transmission at reduced safety factor. Finally, as predicted from the function of Rapsyn, a mutation in *rapsyn* lowers AChR density.

The downregulation of AChR α–,β–, γ–, and δ-subunits in non-synaptic fiber regions by electrical impulse activity implies that a decrease in activity results in an incease in non-synaptic ACh sensitivity, as is the case in numerous clinical conditions lowering mobility. As predicted (see above), hypersensitivity is mediated by the fetal AChR type.

Mode of action of succinlydicholine (suxamthonium) as a neuromuscular blocking agent

By analyzing single channel current events, Colquohoun and colleagues have examined the basis of succinyldicholine's action as a neuromuscular blocking agent. They concluded that succinyldicholine is about 8-fold less potent than ACh, due to lower binding affinity to the receptor and because of a lower ability to activate the channel once bound. At the same time, it can block the open channel. However, in clinical practice, channel block does not appear to to contribute significantly to the neuromuscular block. Rather, paralysis appears to be caused by prolonged depolarization of the endplate region. This leads to inactivation of the voltage-gated Na^+ channels normally eliciting the action potential.

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